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APPLICATION OF FLOATING WETLAND FOR TREATMENT OF PAPER MILL WASTEWATER

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Abstract

The wastewater from the paper mills is described by dark yellowish color, high amounts of COD, phenol, BOD, pH, TDS and lignin. This study concerns plants were vegetated in wastewater of paper mill by using different treatment named as T1, T2, T3, T4, T5 and T6.The examination was run in duplicated with the resulting treatment design (T1) *T. domingensis* + Tap water, (T2) Wastewater and *T. domingensis*, (T3) Wastewater + *T. domingensis*+ M40, (T4) Wastewater + *T. domingensis* +M8, (T5) Wastewater + M40, (T6) Wastewater + M8 in pot tests. As *T. domingensis* is an aquatic species, holds great potential to remediate contaminated water. In this study impact was seen on the effect on growth of *T. domingensis* and the level of toxicity in contaminated was estimated by using Fish Toxicity assay.

Keywords: Wastewater, COD, BOD, PH, TDS, WW

Introduction

Earth is familiar as the "Blue Planet". It covers with water about 71%. The earth has a plenty of water, but 99.7 % is in the form of seas, soils, icecaps, and sailing in the climate and surprisingly, just a little rate (0.3%), is available for living beings. But rapid population growth, industrialization and anthropogenic activities results in water pollution which is accumulated in food webs causing a serious public health risks (Ndiaya and Lv, 2018).

Among various industries, paper industry is the 6^{th} largest industry and key contributor of the water contamination as it releases enormous amount of effluents. It is estimated that up to $70m^3$ of wastewater is released from every metric ton of paper production, depending upon type of crude material, the amount of water used, and the type of end item (Ram et al., 2020).

The discharged effluents results in ecological contamination and huge health problems. The untreated effluent harms the water quality and all life forms. Several studies reported the harmful impacts including physiological changes, respiratory issues, harmfulness and deadly consequences (Sasi et al., 2020)

During paper processing, pulping produces higher amount of wastewater which contains wood trash and their dissolvable materials, lignin, starch and suspended solids. Chlorination is the main stage in kraft dying (Pathak and Sharma, 2021). During this treatment, chlorinated substances are produced which persists in nature for longer period of time.

Lignin and its various types are hard to degrade because of the presence of solid intra-sub-atomic C-C linkages. Some poisonous mixtures like gum acids, unsaturated fats, diterpene alcohols and chlorinated tar acids are also present in p Lignin and their derived compound are highly toxic and are known for increasing levels of carbon, COD and BOD.Acute toxicity, target organ toxicity, skin irritation, and carcinogenic and mutagenesis effects of lignin and phenol-derived compounds are well-known. (Singh et al., 2021). paper effluents (Tong et al., 2016).

Effluents from the paper mill comprises toxic pollutants that can create critical harm to the body organs and organ system, like the nervous, immune, reproductive, circulatory, respiratory, sensory and endocrine systems (Singh and Tripathi, 2020). Some of the contaminants present in effluents are termed as EDCs. EDCs are also called exogenous agents that restrict the transport, synthesis, action, secretion and binding of chemicals in the body that are liable for development of reproductive behavior and to maintain internal stability (Singh et al., 2019). Effluents that share adequate structural similarity with endocrine chemicals are called endocrine disruptors that can bind with endocrine receptors and cause toxic effects on successful development of reproductive system and survival of aquatic life. Benzoic acid found in the wastewater of paper mills caused gastric pain, nausea, allergic reaction and vomiting (Kang et al., 2018).

Biological treatment of the effluent includes utilization of microorganisms with lignolytic properties that can reduce the toxicity of harmful compounds. The microorganisms that included for having lignolytic properties are bacteria, yeast, algae, fungi and actinomycetes (Peyrelasse et al., 2021). Utilization of enzymatic treatment alone or in combination with techniques of physical and chemical has been documented in the literature (Chapman et al., 2018). The enzymatic treatment is a safe and ecofriendly method. Wastewater treatment plants ordinarily utilize aerobic as well as anaerobic biological methods to eliminate impurities in wastewaters. Of which, paper factories really like to utilize aerobic process due to their low cost and simplicity of activity (Ferdeş et al., 2020; Peyrelasse et al., 2021).

Anaerobic processes are not common; various factories utilize distinctive anaerobic processes because of lesser sludge formation, sustainable energy production, covering a small area and degradation capacity of pollutants. Both processes have disadvantages like high sludge formation in oxygen consuming process and sensitivity of anaerobic microorganisms for toxic materials (Barakat et al., 2014).

Ligninase, cellulose and peroxidase have showed potential to eliminate organic compound from paper factory effluents and furthermore playing fundamental part to work on the quality of wastewater (Kumar and Chandra, 2020). Out of them the main catalysts, particularly peroxidase which is utilized for color elimination in effluents. It is likewise conceivable to used mixed enzymes as one with bacterial strain which ordinarily do not have high potential for decolorization of wastewater (Milena, 2020). White rot fungi utilizes glucose as energy source to produce an extracellular compounds that is called peroxidase. This extracellular compound (peroxidase) oxidizes the chromophores and remove the color from effluent by lowering degrees of hydrogen peroxide was improved by (Hosseini Koupaie et al., 2019).

Oxygenases are the oxidoreductases that are utilized in the oxidation of reducing substrate (Arora et al., 2009). These catalysts have a huge role in the degradation of organic material as they increase their reactivity or dissolvability in water or lead to the cleavage of aromatic compound (Arora, 2010).

Laccases are multicopper oxidases made from modified plants, insects and fungi (Elbeshbishy et al., 2017). These microorganisms catalyze the oxidation of phenolic and aromatic compounds with associative reducing of sub-nuclear oxygen from water (Shraddha et al., 2011). Aromatic compounds including phenols and amines, include one of the significant classes of poisons that are present in the wastewaters (Lellis et al., 2019).

During the enzymatic hydrolysis, cellulose can be by the cellulases. Cellulases cause the removal of cellulose microfibrils that are produced during washing process of cotton-based materials (Vasconcelos and Cavaco-Paulo, 2006). In the paper and pulp industry, cellulase is used for the removal of color in reusing of paper. Before 10 years, there has been huge interest for cellulose hydrolysis developed (Kenealy & Jeffries, 2003). This interest is because of their advantages like offering methodology, specifically, the transformation of lignocellulosic and cellulosic waste by the formation of biogas, sugars and ethanol (Capolupo & Faraco, 2016).

It includes the use of microorganisms including algae, fungi, bacteria and enzymes or catalysts for treatment. These microbes are being used with plants for treating waste water (Hossain and Uddin, 2021). This method can possibly kill or decrease the issues related with physicochemical techniques. A few examinations have been done regarding the decolorization and treating the wastewaters through biological techniques (Aghalari et al., 2020).

The Color of paper effluents is because of lignin and tannins which resistant to degrade. This resistance is because linkages of carbon-to-carbon biphenyl (Wang et al., 2018). It is accounted that lignin is biodegradable by some of microorganisms under appropriate ecological environment. Various microorganisms (*Pseudomonas* spp., *Flavobacteria*, *Xanthomonas* spp., *Bacillus spp.*, *Aeromonas* spp., *Cellulomonas* spp., and *Chromobacrtia)* have been accounted for to break down lignin components (Kamali and Khodaparast, 2015).

The white rot fungi (*Basidiomycetes)* that have a functioning lignolytic enzyme degrade lignin and its derived structures. These organisms utilize lignin source as an auxiliary metabolite which is not needed for their development (Howard et al., 2003). The degradation of lignin by this fungus is broadly considered and is brought by three extracellular phenol oxidases that are named as LiP, MnP and laccases (Ayele et al., 2021a). These fungi are likewise fit for degrading chlorinated compounds, heterocyclic phenolic hydrocarbons and their polymers, and different colors (Costa et al., 2017).

White-rot fungi (*P. Chrysosporium)* with white-rot fungi (*P. sanguineus, P. ostreatus and H. annosum*) and with the utilization of the surfactants are another option to remove color, COD, and lignin. Lignin, BOD, COD and color removal were accomplished by *Pencillium* sp (Singh et al., 2019). It is reported that *T. versicolor* had the option to proficiently degrade the soluble substances. it was revealed That white rot fungi like (*Tinctoporia borbonica, Schizophyllum collective, Aspergillus fumigatus, Pleurotus ostreatus*) to degrade color of effluents. Number of bacterial species has been reported for their removing color in effluents and some are being used at commercial level. *Bacillus subtilis* with *Micrococcus luteus* were found for reduction of BOD, COD and lignin content up to 87, 97% respectively following 9 days under shaking setup and decrease the pH and dissolved Oxygen (DO) in the effluent from 0.8-6.8 mg L^{-1} (Chandra & Bharagava, 2013).

The utilization of plants for remediation of water contaminated with metals, has acquired acknowledgment in the beyond twenty years as a minimal expenses and non-invasive strategy (Yan et al., 2020). This methodology is arising as an imaginative tool with positive potential that is most helpful when toxins are inside the root zone of the plants. Moreover, phytoremediation is cost-effective and aesthetically remediation technology with low to moderate degrees of pollution (Bauddh et al., 2017).

The elimination of contaminations relies on the exposure time, amount of toxic compounds, ecological factors (pH, temperature) and plant attributes (species, root system and so on). Furthermore, emerging plants *Typha* spp., *Phragmites autralis Nymphaea* spp., and *Hydrochloa caroliniensis* aquatic plants like *Azolla pinnata, Salvinia molesta, Lemna spp., Pistia stratiotes,* submerged plants such as *Najas marina, corymbosa, Hygrophilla,* and have been utilized for phytoremediation purpose (Ekperusi et al., 2019).

Typha grows near to water, in lakes, ponds and riverine spaces of various districts of the world, in America, Europe and Asia. Southern cattail *(Typha domingensis)* is a flood-tolerant and salt-tolerant specie, a tall (2.0-2.5 m) plant considered as the source of paper and fiber (Rani et al., 2015).

Studies revealed that *Typha* spp. shows high bearing capacity and high toxic substance take-up potential to safe water or soil from contamination (Shehzadi et al., 2014: Hegazy et al., 2011). Floating wetland an effective technology for remediation of wastewater. This method was first time established in Japan and Germany for eutrophic remediation (Shahid et al., 2018). Now this system is being used to treat wastewater of domestic, sewage, contaminated groundwater, nutrients enriched water, poultry effluent, polluted river water and industry effluents. Plants are vegetated hydroponically on floating structure but plant's root is kept down in contaminated water as boil.

Material and Methods

Wastewater Collection and Characterization

Wastewater was collected from the outlet of Century Paper and Board Mills, Lahore by using composite technique. Sample was collected in sterilized plastic bottles and taken to Life Sciences Laboratory at University of Management and Technology, Lahore for further analysis. Effluents were stored at 4°C (Zainith et al., 2019a). Effluent was analyzed for various physico-chemical parameters such as pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), total phenol and electrical conductivity (EC).

Experimental Site and Duration

The present work was done in the green house of the University of Management and Technology, Pakistan from January 2021 to May 2021.

Selection of Plant

Typhadomingensis (southern cattail/cumbungi) was obtained from local nursery Pattoki, Kasur. It is perennial herbaceous plant, belonging to genus *Typha* and found in temperate and tropical regions worldwide. This plant was chosen because it can survive and grow in the harsh environmental conditions of Pakistan.

Bacterial Strains

Four bacteria belonging to different species were selected and screened for their ability to degrade lignin present in wastewater.

Table 1: Bacterial strains used in this study

Sr. No.	Strain Code	Strain Name	Reference
1	N4	*Klebsiella sp.*	NIBGE
2	M32	*Acinetobacter aeruginosa*	NIBGE
3	M8	*Acinetobacter sp.*	NIBGE
4	M40	*Pseudomonas aeruginose*	NIBGE

Determination of Lignin Content in Paper Mill Wastewater

2M H_2SO_4 and 2M NaOH were added in 10 mL wastewater to determine the lignin content in paper mill effluent until completely precipitated. After precipitation, for 15 minutes material was centrifuged at 13000 rpm. Supernatant was disposed of, and got precipitate was then washed with deionized water to neutralize its pH. After drying, weight of the precipitate was recorded (Wang, 2020).

Screening of isolated bacterial strains for Lignolytic enzyme activity

The screening of purified bacterial isolates was carried out based on the dye decolorization method.

Preparation of Phenol Red

To prepare the phenol red, take 1000 mL distilled water; add 0.80g of NaOH and 0.1g of phenol red color in it. Blend this mixture with the help of stirrer and stored it in dark place.

Preparation of Mineral Salt Medium (MSM)

The concentration of 0.01% of phenol red dye was put to the sterile MSM under sterile conditions. To prepare 1L MSM, Na_2HPO_4 2.4g, 1.3g KH_2PO_4, Peptone 5.0g, D-glucose 10g, $CaCl_2$ 0.01g, $MgSO_4$ 0.01g, NH_3NO_3 0.1g and 2% agar was added in distilled water.

The strains were assessed for lignolytic enzyme activity using phenol red dye as a marker. Four strains were streaked on MSM agar plates retained with phenol red dye and incubated at 37°C for 48h. A decolorization zone encircled the bacterial strains was the indicative lignolytic enzyme activity (Zainith et al., 2019b).

Green House Experiment

Compatibility Test to Formulate the Bacterial Consortium

After screening of lignolytic enzymatic activity, positive strains were further tested for their ability to grow in combination. To evaluate this effect, one strain was spread on MSM agar plate and other strain was spot inoculated/streaked and incubated at 37℃ overnight. After 24h, plates were analyzed for any clearing zone around the colonies. Clearing zone is the indicative of no compatibility among the strains.

Manufacture of FTWs and Experimental Setup

The macrocosms arrangement was involved six tanks with 11 liter limit each, and the aspects were 23.8cm (L × W × H). The mats were created from extended polystyrene (EPS)- based sheets produced by Diamond® Foam Private Ltd., Pakistan. EPS sheets are inflexible, have low conductivity, are dampness safe and comprise of non-permeable closed cell froth. The size of the mats was adjusted so they could fit in each tank with 2.1 (L×W)>95% inclusion on the water surface. Each of the four sides of the mats was wrapped with aluminum foil to shield the sheets from sun and water harm. In each sheet one equidistant hole were made for the estate of macrophytes on the mats. Each opening was planted with two T. *domingensis*. Each plant gauged 45 to 65 g and their length was 55-65 cm. The plants were permitted to fill in water for one month to acquire ideal development of roots and shoots. Following one month, the normal tallness of the plants was around 145 cm, and the new water in tanks was replaced with paper mills Wastewater. The examination was run in duplicated with the resulting treatment design:

T1: *T. domingensis* + Tap water

T2: Wastewater and *T. domingensis*

T3: Wastewater + *T. domingensis*+ M40

T4: Wastewater + *T. domingensis* +M8

T5: Wastewater + M40

T6: Wastewater + M8

1% inoculum was added in the respective treatments. The experiment was performed for 2 months until color and pollutants were removed from the treated water. After 60 days, samples were collected and put away in a cool and dry spot for analysis such as pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), total phenol and electrical conductivity (EC) according standard method (Apha et al., 2012). The evapo-transpiration losses were recuperated by pouring new water in treatment tanks up to the degree of 11L in each tank. If there should be an occurrence of downpour, the tanks were covered with plastic sheets.

Plant Biomass

To decide the impact of bacterial inoculation and toxicity on plants development and improvement, the information about plants agronomic parameters (root and shoot length and dry biomass) were noted toward the finish of the test. The root and shoot length was estimated manually by an measuring scale. The root and shoots were gathered close to the outer layer of the mat and oven dried at the 80°C for 72 h until a consistent weight was accomplished.

Estimation of Toxicity Using Fish Toxicity Assay

Treated effluent was collected from each tank at the end of this experiment for evaluating its toxicity as described earlier (Ashraf et al., 2018). In short, three (*Labeorohita*) healthy fish was exposed in each treated effluent of the paper mills. The quantity of fish that passed on was counted each 24 h for 4 days.(Karstens et al., 2021).

Results

Physico-Chemical Analysis of Wastewater

The physico-chemical analysis of untreated paper mill effluent are mentioned in **Table 3.1**.The pH value of the effluent was significantly higher (pH 8.1) i.e. slightly alkaline. The EC (2.45 ms), BOD (426 mg/L), COD (741.32 mg/L), TDS (1220), lignin (1850 mg/L) and total phenol content (39.3 mg/L) was measured using standard protocols.

Table 2: Physico-chemical properties of wastewater from paper mills

Parameters	Concentration in untreated paper mill effluent (mg/L)
pH	8.1
BOD	426
COD	741.32
TDS	1220
Lignin	1850
Phenol	39.3

The bacterial strains were assessed for lignolytic enzyme activity using phenol red dye as a marker. Five bacterial strains were streaked on MSM agar plates amended with phenol red dye and incubated at 37 °C for 48h. A decolorization zone around the bacterial strains M40 and M8 was the indicative lignolytic enzyme activity.

Compatibility test to formulate the Bacterial consortium

Positive strains were further tested for their ability to grow in combination. To evaluate this effect, one strain was spread on MSM agar plate and other strain was spot inoculated/streaked and incubated at 37°C overnight. After 24h, plates were analyzed for any clearing zone around the colonies. Clearing zone was observed around the bacterial colonies M8 and M40. So it is concluded that these strains are not compatible with each other and can be used independently.

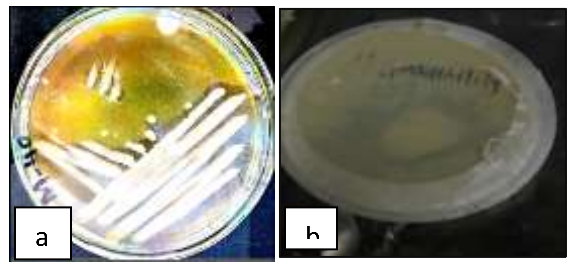

Figure 1: a) Screening of lignolytic enzyme activingon MSM agar plate amended with phenol red dye (100 ul/L) b) Screening of Bacterial Strains for Ligninolytic Enzyme Activity

Plant Growth Responses to Bacterial Inoculation

Growth variables (root and shoot length, root dry weight and stem dry mass) were recognized to assess the impact of inoculation of bacteria on plant. In water contaminated with papers mill effluents, bacterial inoculation (M8 and M40) improved root length (87%), shoot length (25%), root dry weight (72.54%), and shoot dry weight (30.94%). However, plants inoculated with M40 showed more expansion in root length (5%), shoot length (20%), root dry weight (13%) and shoot dry weight (20%) when contrasted with M8.

Effect of Plant Species on Wastewater Treatment

T. domingensis reduced pH (50%), BOD (87%), COD (86%), TDS (61.94%), total content of phenol (51%), lignin (45%) and EC (57.44%)

value of the effluent was observed in treatment having plants inoculated with bacteria. Vegetated systems exhibited more wastewater contamination reduction (60-66%) than unvegetated treatment; whereas the augmentation of unvegetated system with inoculated bacterial strains resulted in 40% reduction. On the other hand least degradation was detected in that control that was uninoculated and unvegetated.

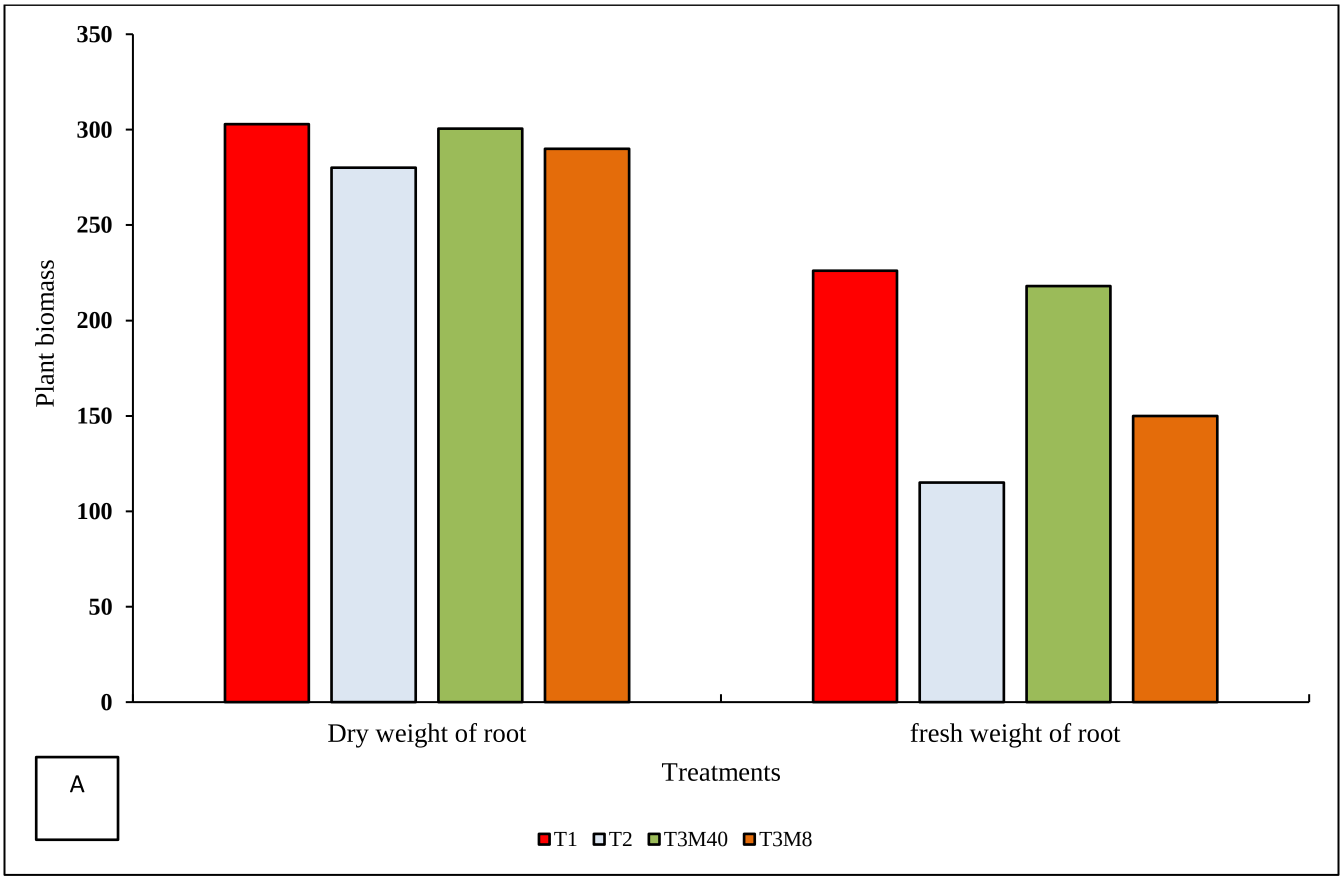

Figure 2 (A) Comparison of *Typha domigensis* growth

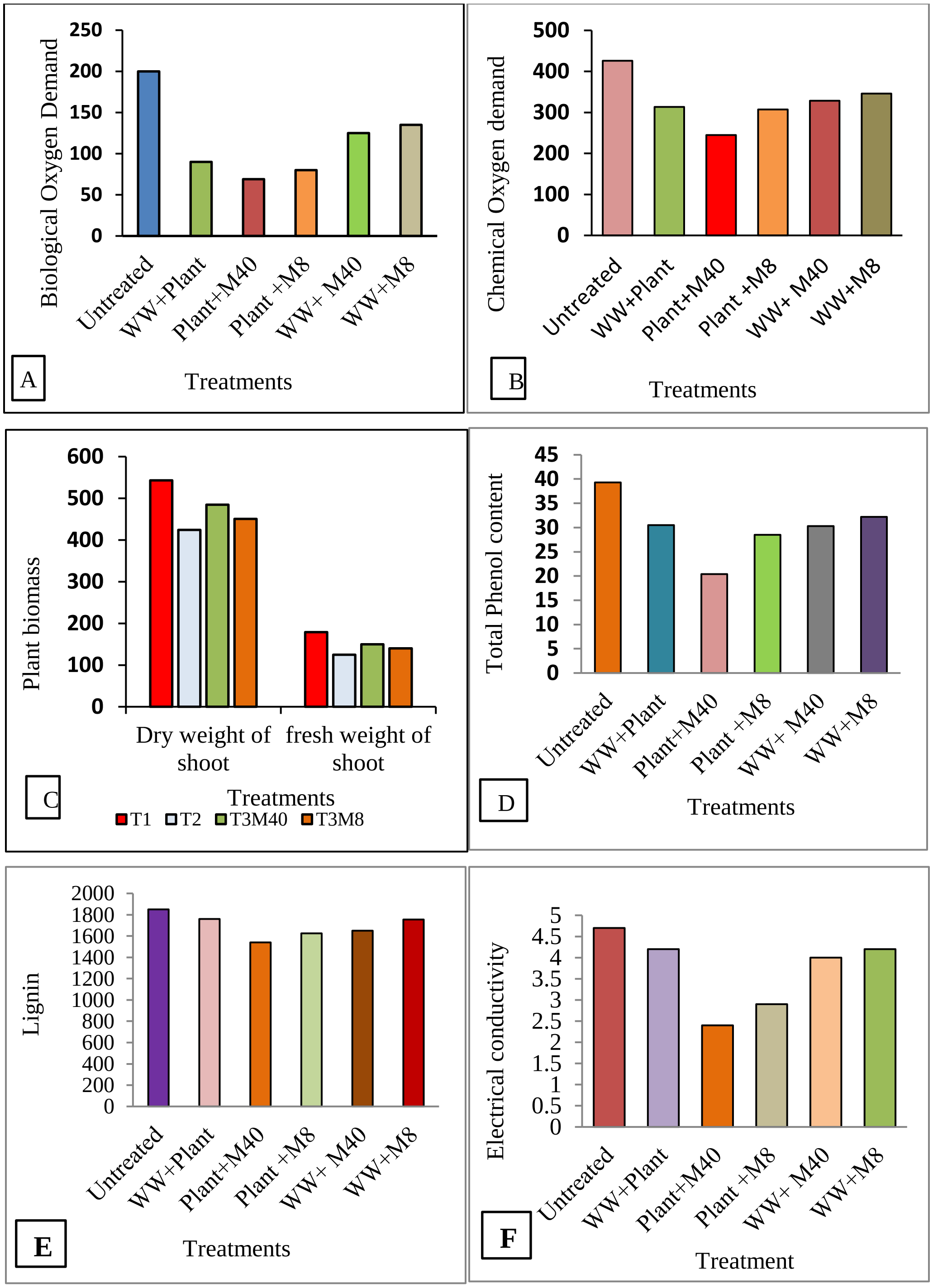

Figure 0: (A) BOD (B) COD (C) TDS (D) Total phenol content (E) lignin (F) EC: reduction in paper mills wastewater. T-2 (*Typha domingensis* and WW), T-3 (*T. domingensis* and M40), T-3 (*T. domingensis* and M8), T-4 (*T. domingensis* and M40) and T-4 (*T. domingensis* and M8).

Estimation of Toxicity using Fish Toxicity Assay

Treated effluent was collected from each tank for evaluating its toxicity; the method was used to check the behavior of fish for treated wastewater. It was observed from result that fish was able to survive in this treated water except control T1.

Table 0: Mortality in fish in paper mills wastewater T4 on exposure to different concentrations of treated effluent by CWs using *Typha domingensis.*

Treatments	Survival Rate	Death Rate
T1	3	0
T2	1	2
T3	3	0
T4	2	1
T5	1	2
T6	1	2

Discussion

The untreated physic-chemical analysis of paper mill effluents is mentioned in table 3.1.

The treatment of phytoremdiation paper mill's wastewater by using *T.domigensis* improved the uality of paper mill's wastewater by lowering the pollution rate. The pH value of effluents was significantly higher (pH 8.1) i.e. slightly alkaline with EC (4.7ms), BOD (200mg/L), COD (420mg/L), TDS (1220), lignin (1850mg/L) and total phenol (39.3mg/L). The concentration of pollutants in wastewater is highly variable and is industry specific (Ilyas etal., 2019). The higher value of pollutants in paper mill effluent as shown in Table 2 made our environment toxic by changing the composition of ions and raised its salinity (Singh et al., 2019).

The quality of wastewater improved significantly after bacterial treatment process. The satisfactory result was observed by combination of *Typha domigensis* with M40 strain than M8. The level of COD was reduced 87% in T3 M40 in comparison to 62% wasin T2. Similarly from an initial value of 200 mg/L, minimum BOD of 69 mg/L wasreached by utilization of plant with M40 Figure 3.3 (a) exhibited better reduction of pollutant in T3 in comparison T2. These initial values are higher in paper effluents due to presence of lignin and its derived compound, fibres, chlorine and its derived compound, resin acid, tannins, fatty acid, sulphur and its derived compound and lower level of DO (Jaiswal et al., 2021). From preliminary data on wastewater showed that lower value of DO in wastewater is due to bacteria and higher amount of BOD. The level of free Oxygen, which does not gradually increased in wastewater is referred as DO (Hegazy et al., 2011). It is a main parameter in estimating water quality. It can endangered the aquatic life and affect water quality if value is excesses or to be lowered. The lowest value of BOD shows a good quality of water, while higher value of BOD indicated the water is contaminated. The reduction is because of plant' root system, plant with fibrous roots is able to accumulate more pollutants in comparison to taprrot containing plants. The growth of plant root increased than shoot of plant in wastewater (Aziz et al., 2020). From literature, it was concluded *T. domingensis* shows metal concentration in plant tissues higher ($p<0.05$) than control sample *Typha domingensis* accumulates the pollutants in their root in comparison to shoot. So, *T. domingensis* is a good pollutants accumulator (Hegazy et al., 2011). The reduction of COD and BOD was calculated because plant subjected to do photosynthesis. This activity increased the DO level in water. It is an indication that now this wastewater that treated with ecofriendly technology phytoremediation would have less harmful impact on environment. The aforementioned positive outcomes obtained for decrease in contamination parameters by utilization of vegetation and related microscopic organisms are confirmed by ongoing reports also (Ijaz et al., 2015) utlilized *Brachiaria mutica* and endophytes *(Bacillus licheniformis, Bacillus cereus, Acinetobacter sp.,)* on sewage effluents to obtain decrease COD and BOD, with in 72h. Similarly (Shehzadi et al., 2014b) treatment of wastewater containing toxic compound utilizing FTWs vegetated with *Typha domingensis* and inoculated with plant promoting endophytes to report a derease COD, BOD, TDS and TSS. A complete pattern was noticed for physicochemical parameters, such as EC, pH, anf for lignin. The pH was seen to move toward alkalinity (7.9). This might be due to production of organic acid by degradation of pollutants. This degradation was further increased by M8 and M40 bacterial strains was just due to having degradation ability as described earlier (Tusief et al., 2019).

EC also measured lower from 4.70 to 2.45mS/cm as shown in Figure 3.4 (f). EC is a significant natural quality parameter for water intended to reuse for irrigation. With an initial value of EC that was 4.7mS/cm lowered due to reduction of salt by up taking root of plant. Additionally, bacterial strains boosts this property of up takes the salts through root (Tusief etal., 2019). The amount of lignin in wastewater of paper mills before and after different treatment T1, T2, T3, T4. The content of lignin is decreased from its initial value as shown in Figure 3.4 (e). The efficiency of lignin removal is nearly 90% as described in previous study (Grismer and Shepherd, 2011). The basic factor for reduction of toxic pollutants is due to increase in Do; presence of *T. domingensis* enhance the level of O_2 . Fish were found deceased in waste water, which suggested that T. domingensis in T2 was unable to completely build up the effluents. So, in case of control T2, fish survival was seen less than half. However, no fish was dead in T3M40 indicating the possibility of 90% removal by combining plant and bacterial strains (Aziz et al., 2020).

Conclusion

In this study, wastewater of paper mills that inhibits plant growth due to lower level of DO and presence of heavy metal and toxic compound drastically affect plant photosynthesis rate, plant biomass, evapotranspiration and metabolic function. As a result toxic compound begin to start accumulation and damage the plant defense system. That is why biomass decrease and less effective reduction of lignin, BOD, COD, EC and phenol was observed in T2. On the other hand, T3M40 shows the higher biomass than T3M8. The combination of plants and contaminant degrading microbes for cleanup of environment environmental pollutants has gotten a lot of consideration in recent years. This study aims to utilized the plant microbe association for the removal of lignin, COD, BOD and phenol from paper mill wastewater. Two isolated bacterial strains namely (M8 and M40) were inoculated with *T. domingensis* for improving water quality in FTWs reactor.

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